

## Review article

## Update on the evaluation of hypersensitivity reactions to betalactams

Hypersensitivity reactions to betalactams (BLs) are classified as immediate or nonimmediate. The former usually appear within 1 h of drug-intake and are mediated by specific IgE-antibodies. Nonimmediate reactions are those occurring more than 1 h after drug-intake, and they can be T-cell mediated. The diagnostic evaluation of allergic reactions to BLs has changed over the last 5 years, for several reasons. Major and minor determinants are no longer commercially available for skin testing in many countries. In immediate allergic reactions, the sensitivity of skin testing and immunoassays is decreasing and new *in vitro* methods, such as the basophil activation test, are gaining importance for diagnosis. For nonimmediate reactions, skin testing appears to be less sensitive than previous results, although more studies need to be carried out in this direction. Nevertheless, the drug provocation test is still necessary for diagnosis.

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Allergy to betalactams (BLs) continues to be the most frequent cause of drug reactions mediated by specific

immunological mechanisms, and it can be induced by all BLs currently available (1, 2). Benzylpenicillin (BP), the structure inducing sensitization, has been progressively replaced by amoxicillin (AX) and to a lesser extent by cephalosporins or other BLs (1, 2). Increasing evidence supports the role of the side chains as the relevant part of the structure of the allergenic determinants (3).

On the basis of the time of appearance of the reaction after drug-intake and for diagnostic purposes, hypersensitivity reactions to BLs have been classified as immediate

**Abbreviations:** AGEP, acute generalized exanthematous pustulosis; AX, amoxicillin; BAT, basophil activation test; BLs, betalactams; BP, benzylpenicillin; BPO, benzylpenicilloyl; DRESS, drug reaction with eosinophilia and systemic symptoms; DPTs, drug provocation tests; ENDA, The European Network for Drug Allergy; LTT, lymphocyte transformation test; MDM, minor determinant mixture; MPE, maculopapular exanthema; PPL, benzylpenicilloyl-polylysine; RAST, radioallergosorbent test.

or nonimmediate. The former usually appear within 1 h of drug-intake and are mediated by specific IgE-antibodies. Nonimmediate reactions are those occurring more than 1 h after drug-intake and can be T-cell mediated (4, 5).

The European Network for Drug Allergy (ENDA) has devised various diagnostic algorithms for the evaluation of immediate (4) and nonimmediate reactions (5). However, the evaluation of allergic reactions to BLs continues to pose difficulties, for several reasons, including the fact that skin testing with the classical penicillin reagents, major and minor BP determinants, is losing sensitivity and also that these reagents are no longer available everywhere. In addition, *in vitro* tests for IgE determinations need to be improved and cellular tests to detect either IgE- or T-cell mediated responses are not fully validated nor routinely used because of their technical complexity. The present review aims to improve the evaluation of BL-allergic patients, with the inclusion of recent data.

## IgE-mediated reactions

### Mechanisms

Betalactams need to be conjugated to carrier molecules to induce sensitization and a clinical response after subsequent exposure, although the details of the protein-binding and processing have not yet been fully determined. Conjugation of the BP via the carbonyl group induces the benzylpenicilloyl determinant (BPO) (6). Other structures to be considered are benzylpenicilloic, benzylpenicillenic, benzylpenamaldate, benzylpenaldate, benzylpenicoil and benzylpenicilanyl (7), although their clinical relevance has yet to be shown.

The role of the side-chain structures as antigenic determinants is now widely accepted, particularly in hypersensitivity reactions to AX and cephalosporins (8–16). In the latter, after the opening of the BL ring by the amino group of the carrier, the R2 side-chain can disappear and a heterogeneous fragmentation of the cephalosporin occurs (14, 17, 18). The final product of the epitope is therefore formed by the remaining BL and the R1 side-chain. The role of R2 side-chains as allergenic determinants has mainly been shown *in vitro* and a detailed analysis of the relevance of this is beyond the scope of this review (19).

The antigenic determinants have been used for diagnostic purposes by *in vivo* or *in vitro* testing, although these approaches are not equivalent. In skin testing, while some determinants, such as BPO, need to be conjugated to a carrier molecule, polylysine producing benzylpenicillin-polylysine (PPL) (6), to induce a positive response. Others, such as the minor determinant mixture (MDM), BP, AX or different cephalosporins, can be applied free. However, for the detection of specific IgE antibodies *in vitro*, the determinants need to be conjugated to a carrier, such as polylysine, human serum albumin (HSA)

or aminospacers (20). For the basophil activation tests (BAT), a higher sensitivity has been demonstrated with unconjugated drugs (21).

### Clinical patterns

The two main entities usually recognized are urticaria, with or without angioedema and anaphylaxis. Urticaria is defined as rapidly evolving transient pruriginous wheals occurring at different sites of the body, which may represent the first stage of an anaphylactic reaction. Anaphylaxis is defined, according to Sampson et al. (22), as a serious allergic reaction with a rapid onset that may cause death; it should be diagnosed by the clinical criteria proposed by the aforesaid authors (22). Allergic reactions to BLs manifest predominantly as bronchial asthma and/or rhinitis, which can be explained by special sensitivity in a previously affected organ. These entities can also occur as occupational respiratory diseases.

### Diagnostic methods

The first approach to diagnosis is to establish a plausible history, which implies that symptoms must be compatible and occur within 1 h of drug-intake. This is relevant because many differences between published studies are related to patient selection, the lack of differentiation between immediate and nonimmediate reactions, or even to misclassifications. Fulfilment of the ENDA questionnaire reduces differences in evaluation (23). The diagnostic tools available below.

**Skin tests.** Skin testing is the basic diagnostic tool, although in patients with a history of severe reactions, *in vitro* tests may be the recommended choice. The general rules in the ENDA document (4) are still valid, although new data warrant a revision of a few specific points:

**Withdrawal of major and minor determinants.** In both the ENDA document (4) and the American practice parameters (24), skin testing with the classic BP reagents (PPL and MDM) represents the first-line method for diagnosing immediate hypersensitivity reactions to BLs. However, after Allergopharma and Hollister-Stier ceased production of PPL and MDM in 2004, the diagnostic evaluation of immediate reactions to BLs had been hampered. This had led to several publications recommending either desensitization or performance of drug provocation tests (DPTs) without skin testing. These procedures are not always appropriate and may be risky. Recently, PPL and MDM have been commercialized by another company (Diater, Madrid, Spain), and studies carried out in Europe, Australia and New Zealand indicate that they are equivalent to those from Allergopharma (25–27). No other commercially available alternatives are available so far, although some groups have made their own conjugates (28).

**Haptens:** It is difficult and even arguable whether to recommend a fixed panel of haptens to be included in the diagnostic evaluation, but some reagents are essential. The ENDA group considers that PPL, MDM and AX are the first determinants to be tested, particularly in persons with immediate reactions to unknown BLs, in addition to the suspected determinants. These reagents should be used in either the short (Fig. 1) or the long algorithm (Fig. 2). Because recent data (29) suggest that the use of BP up to a concentration of 10 000 IU/ml in skin testing may increase the allergological work-up sensitivity, it is recommended that in case of negative results to the classical penicillin reagents (PPL and MDM), BP should also be tested. Finally, subjects who have experienced immediate reactions to the combination of AX plus clavulanic acid and show negative results in the diagnostic work-up should be skin tested with this combination because a few selective reactions to clavulanic acid have been reported (30), even though clavulanic acid alone is not commercially available. The haptens and concentrations recommended are shown in Table 1.

**Sensitivity and specificity.** The percentage of positive responses may vary, not only in different populations, but also over the years. In studies performed in the same population, 77.7% of the patients initially presented positive responses to skin tests with PPL and/or MDM, whereas this percentage fell to 42.1% and 22.1% over the years (8, 31). PPL and/or MDM have recently been reported to induce a positive response in 46% of patients with a positive skin test, independently of the skin test response to the culprit drug, with 14% being exclusively positive to both haptens (32). The decrease in the sensitivity of PPL and MDM has been progressively

compensated for by an increase in the sensitivity of skin testing with AX. The specificity for these haptens has not changed over the years. Likewise, elective penicillin skin testing in children revealed a marked decline in its sensitivity (33).

Concerning cephalosporins, skin test sensitivity in two recent studies varied from 30.7% (39 out of 127 persons) (14) to 69.7% (53 of 76) (16). Therefore, further studies in larger numbers of subjects are required to fully establish cephalosporin skin test sensitivity.

**Resensitization after allergological tests.** In some studies (34–36), resensitization (i.e. a conversion to skin test positivity) has been observed in patients retested about 1 month after a negative allergological test, including DPT, followed by full therapeutic courses, with a frequency ranging from 0.9% (34) to 27.9% (37). Specifically, in a recent study (37), skin testing and DPTs with different BLs were performed prospectively in patients with a history of remote penicillin allergy, with a rate of conversion to skin test positivity of 27.9%. This phenomenon appears to occur mainly in subjects who have experienced immediate reactions to BLs. However, the aforementioned studies (34–36) evaluated patients with either immediate or nonimmediate reactions. If we just consider patients with immediate reactions, the rate of conversion to skin test positivity could be higher, as has recently been observed in a small number of patients with immediate reactions to cephalosporins (16). Bearing these data in mind, it seems advisable to retest patients who suffered immediate reactions and who displayed negative results at the first allergological evaluation, including DPTs.

The question of establishing whether selective responders can be sensitized to closely-related BLs at subsequent

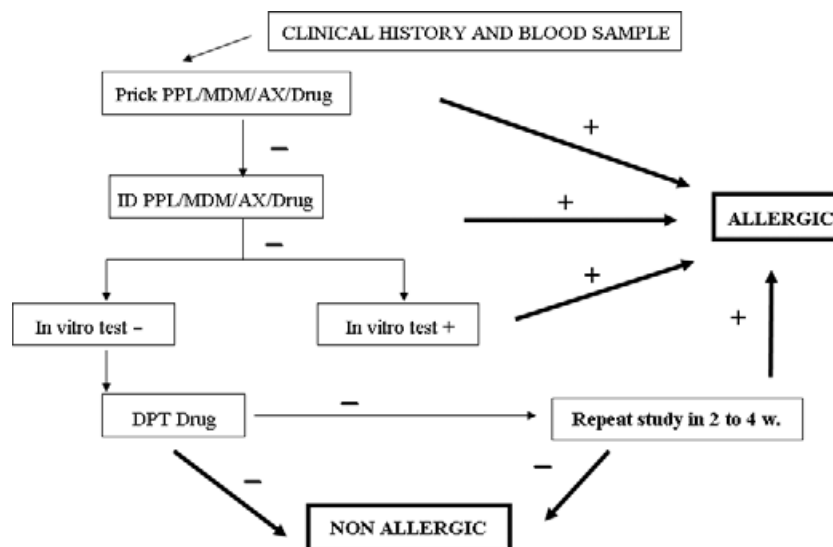


Figure 1. Short algorithm for the diagnosis of immediate allergic reactions to betalactams. This algorithm has the advantage that it can be performed in 1 day and the disadvantage that it fails to distinguish the selectivity of the reaction.

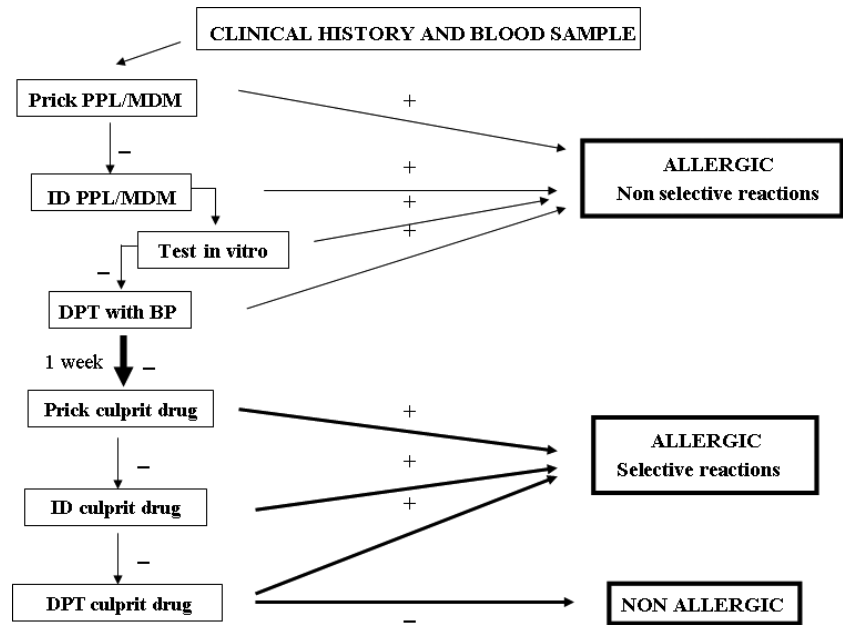


Figure 2. Long algorithm for the diagnosis of immediate allergic reactions to betalactams. This algorithm has to be performed over two separate days but it has the advantage that it distinguishes the selectivity of the reaction.

exposures is important. Recently, the repeated administration of BP and penicillin V in subjects with selective responses to AX failed to induce any change in the rate of negativization compared with a control group, with no appearance of new sensitizations to BP determinants (38). This could be extrapolated to other not-so-closely-related chemical structures, such as cephalosporins, although detailed follow-up studies are required in this context.

**Systemic reactions after skin testing.** A rate of 1.3% of generalized reactions in all tested patients and 8.8% in patients with a positive skin test has recently been reported (39), which is similar to other published data (31). This study (39) confirmed that a history of anaphylaxis was indeed a risk factor. Considering these observations, certain precautions should be taken during BL skin testing to prevent systemic reactions: ready-to-use emergency material, consideration of contraindications (beta-blockers), starting with low and then

gradually increasing concentrations, and strict supervision in the hospital during and after (at least 1 h) skin testing. In patients at risk, skin tests with the different determinants should be separated by sufficient period of time.

**In vitro tests.** Two main methods have been used to detect specific IgE antibodies to BLs: detection of these antibodies in serum by immunoassays and detection on the basis of basophil activation on contact with the hapten, either by quantification of mediator release (histamine or leukotrienes) or basophil activation marker expression. Different studies over the last 10 years have shown variable concordance with *in vivo* tests, with some cases only positive *in vitro*. All these techniques are accepted to be complementary and in some cases, for example in patients with severe reactions, the *in vitro* test should be the first choice in the diagnostic evaluation.

**Immunochemical assays.** Although for many years a number of groups used their own methods to assess specific IgE antibodies, commercially available kits are now more suitable for routine analysis (4). The Phadia CAP System FEIA method (Phadia, Uppsala, Sweden) is on the basis of high surface capacity solid phase to which the hapten is bound by a spacer and a secondary fluorolabelled antibody, with a specific IgE measuring range of 0.01–100 kUA/l, a cut-off value of 0.35 kUA/l for positive results, and levels higher than 0.10 kUA/l indicating sensitization. The overall sensitivity in 48 patients with skin test positivity to AX- and/or BP-derived agents was 54%, with a specificity of 95%

Table 1. Haptens and the highest concentrations recommended for prick and intradermal tests

Hapten	Dose	Unit
PPL	$5 \times 10^{-5}$	mMol/l
Minor determinant mixture	$2 \times 10^{-2}$	mMol/l
Amoxicillin	20	mg/ml
Benzylpenicillin	10 000	IU/ml
Culprit drug		
Cephalosporin	2	mg/ml
Amoxicillin-clavulanic	20	mg/ml
Ampicillin	20	mg/ml



to 100% (40). Another similar study found that the sensitivity and specificity of this FEIA method were lower, 37.9% and 86.7% respectively (41).

Comparison of this technique with a home-made radioallergosorbent test (RAST) found that the specificity of CAP-FEIA ranged from 83.3% to 100% and the sensitivity from 12.5% to 25%, depending on the clinical manifestations, being higher in anaphylactic shock. The specificity of RAST was from 66.7% to 83.3% and the sensitivity from 42.9% to 75%. In the subgroup of patients with an anaphylactic shock and negative skin tests, the sensitivity and specificity of RAST were 75% (42).

Nowadays, the specificity of methods that measure specific IgE to BLs is acceptable, but the sensitivity needs to be improved, especially in those patients with a clinical history of anaphylactic shock and negative skin tests. Nevertheless, cases with a history of an immediate reaction to BLs, displaying negative results in skin tests and positive results in serum-specific IgE assays, have been reported (11, 14, 31, 40). It is therefore advisable to perform serum-specific IgE assays in addition to skin tests in order to improve the sensitivity of the allergological work-up.

**Cellular tests.** Different tests on the basis of the capacity of basophils to be activated and release different mediators have been developed.

The cellular allergen stimulation test quantifies the sulphidoleukotrienes (LTC<sub>4</sub> and its metabolites LTD<sub>4</sub> and LTE<sub>4</sub>) produced and released upon *in vitro* drug stimulation of blood leukocytes (mainly basophils) (41). The sensitivity and specificity of this test is too low for it to be recommended as a routine laboratory method.

The BAT is on the basis of the flow-cytometric evaluation of CD63 on the surface of blood basophils, a marker that appears in activated basophils following incubation with drugs or other allergens *in vitro*. Several studies (21, 41) have shown that the sensitivity of the technique is nearly 50% and the specificity 93.3%, the sensitivity being 50.9% in patients with positive skin tests, 60% in patients with negative skin tests and positive immunoassays, and 14.3% in patients with negative skin tests and immunoassays but positive DPTs. A European multicentre study reported similar results. The rate of negativization of specific IgE antibodies by BAT and immunoassay was higher for BAT when compared with the RAST assay (43).

As the last position paper of the ENDA group, immunoassays remain an important diagnostic method and BAT has emerged as a useful tool for the diagnosis of immediate allergic reactions to BLs, especially cephalosporins, for most of which immunoassays are not available. The sensitivity of the BAT has been shown to be 77% (21).

**Drug provocation tests.** Recent publications have reinforced the need to perform DPT in the diagnostic

Table 2. Haptens and doses recommended for drug provocation tests

Drug	Benzyl penicillin	Penicillin V	Amoxicillin
Dose	10 <sup>3</sup> IU/ml	5 mg	5 mg
	10 <sup>4</sup> IU/ml	50 mg	50 mg
	10 <sup>5</sup> IU/ml	150 mg	100 mg
	5 × 10 <sup>5</sup> IU/ml	200 mg	150 mg
			200 mg
Cumulative dose	6 × 10 <sup>5</sup> IU/ml	400 mg	500 mg
Route	i.m.	Oral	Oral
Interval (min)	45–60	45–60	45–60

work-up of patients with allergic reactions to BLs (44, 45). A study by Bousquet et al. (45) evaluated 257 patients with BL allergy and found that 69.3% were diagnosed by skin testing and 30.7% by DPTs. The DPT is still needed to confirm the diagnosis and has to be performed in patients with a suspected drug allergy, in accordance with the ENDA recommendations (4). The recommended doses can be simplified as shown in Table 2. A lower starting dose should be used in patients with severe reactions and the cumulative dose has to be adapted for children or in persons with kidney or liver disease.

As stated in the previous position paper, DPTs with BP can be performed not only in order to establish the responsibility of this drug, but also to distinguish those patients with selective reactions from those who react to the whole penicillin group. DPTs are also useful to assess cross-reactivity between different BLs (4).

#### Assessment of cross-reactivity

The evaluation of cross-reactivity between penicillins and cephalosporins has been carried out by selecting more or less well-defined groups of patients who were originally allergic to penicillins and determining whether they could safely take cephalosporins, with variable results (46). Considering cephalosporins by classification, all first generation cephalosporins have the potential for cross-reactivity, probably because their structural features are more similar to those of penicillin, while most second and third generation cephalosporins are highly unlikely to be associated with cross-reactivity, owing to differences in their chemical structure (14, 18). As the description of side-chain-specific reactions, cases with a selective response to AX have been shown to have a greater cross-reactivity with cephalosporins sharing the same side-chain. A study by Torres et al. (47) showed that 38% of subjects with a selective response to AX developed cross-reactivity with cefadroxil, which shares an identical side-chain at the R1 position with AX. Other studies (48, 49), which evaluated patients with a well-demonstrated IgE-mediated hypersensitivity to penicillins by skin testing with several cephalosporins, found a rate of cross-reactivity of up to 12%. On the basis of these findings,

cephalosporins with a similar side-chain should be avoided in patients with IgE-mediated reactions to penicillin.

It is difficult to determine the role of skin testing in the assessment of cross-reactivity. Although it is accepted that a positive cephalosporin skin test in patients allergic to penicillins indicates cross-reactivity, controversy exists about whether a negative cephalosporin skin test in these patients will predict good tolerance to the cephalosporins concerned. In a study of 128 patients with a well-established IgE-mediated allergy to penicillin (49), all 101 patients with negative skin tests for cefuroxime, ceftazidime, ceftriaxone and cefotaxime who underwent graded challenges with cefuroxime axetil and ceftriaxone tolerated them. However, cases have been reported with selective allergic reactions to AX, with negative responses to skin testing with cephadroxil but a positive response to DPT with cephadroxil (47).

It is important to note that *in vitro* cross-reactivity does not mean that the patient will have clinical cross-reactivity. For example, subjects with a selective reaction to AX and good tolerance to BP have some degree of IgE recognition to BP in immunoassays and also in competition assays (50–52). The same can be applied to BAT (21, 41).

Cross-reactivity is not a bilateral phenomenon, with subjects primarily sensitized to penicillins equally recognizing cephalosporins or *vice versa*. Patients who are evaluated after an immediate allergic reaction to cephalosporins can show one of three different patterns of response: patients who respond to cephalosporin determinants and are also skin test positive to penicillin determinants, and who may therefore have a clinical reaction; patients who respond to cephalosporin determinants, but who are negative to penicillin determinants and tolerate BP; and patients with a selective response to a particular cephalosporin (10, 14, 16). Cross-reactivity between cephalosporins must be considered in terms of the similarity of the chemical structure of the R1 side-chain. Important cross-reactivity has been detected between ceftriaxone, cefotaxime and cefepime, which share an identical side-chain at the R1 position (10), as well as between the aforesaid three cephalosporins and cefuroxime and ceftazidime, which have similar R1 side-chains (10, 14, 16). Nevertheless, some patients have cross-reactivity between cephalosporins with different side-chains, probably because the specific antibodies are directed to the common chemical structure shared by all (10, 14, 16, 18, 19, 38).

Regarding other BLs, two recent studies found a 0.9% rate of positive responses to skin tests with imipenem/cilastatin and meropenem among 112 and 104 adults, respectively, with a well-demonstrated IgE-mediated hypersensitivity to penicillin (53, 54). More recently, another study found a 0.9% rate of cross-reactivity to meropenem in 108 children with penicillin allergy (55). In patients with penicillin allergy, good tolerance to aztreo-

nam has been observed (56), although cross-reactivity with ceftazidime, which shares the same side-chain, has been observed (57).

Thus, the ENDA position for the administration to penicillin-allergic patients of an alternative BL is that once an immediate penicillin allergy has been diagnosed, skin testing with the alternative BL (cephalosporin, carbapenem, aztreonam) is mandatory and, if negative, the relevant drug should be given in an appropriate setting at increasing doses.

## T-cell-mediated reactions

### Mechanisms

The most frequent reactions associated with BLs are maculopapular or morbilliform exanthemas (MPE) (58). In this type of reaction, a predominance of CD4<sup>+</sup> T cells has been found in the perivascular dermis and similar levels of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the dermoepidermal layer and epidermis (59). These cells are activated and express high levels of the skin homing-receptor, cutaneous lymphocyte antigen, and produce cytotoxic markers, such as perforin and granzyme B (60, 61). The cytokine and chemokine pattern is mainly Th1 (IFN- $\gamma$  and TNF- $\alpha$ ), although some authors have indicated that IL-5 may be involved in MPE (59, 62).

In other important entities, such as acute generalized exanthematous pustulosis (AGEP), T cells and neutrophils are involved (63). The pathogenesis of Stevens-Johnson syndrome and toxic epidermal necrolysis has been related with epidermal keratinocyte apoptosis, produced by T cells via two pathways, Fas/Fas ligand or perforin-granzyme B (64). The pathophysiology of drug reaction (or rash) with eosinophilia and systemic symptoms (DRESS) includes eotaxin and IL-5 production, which is important for eosinophil recruitment.

### Clinical patterns

The identification of a nonimmediate reaction to BLs is sometimes difficult, because of the heterogeneity of the clinical manifestations that can be quite similar to the symptoms of infectious or autoimmune diseases. These reactions may be manifested by a concomitant viral infection, such as those caused by human immunodeficiency virus, cytomegalovirus, human herpes virus 6 or Epstein-Barr virus (65). Although a high proportion of subjects with exanthematous reactions have good tolerance to the BLs concerned a few weeks after resolution of the viral infection (66), some develop a new reaction after BL re-exposure in the absence of the viral disease (67); in these circumstances they are considered as true allergic. The incidence in children is even lower, probably because of a lower cumulative exposure or to a faster loss of sensitivity (68).

### Diagnostic methods

**Skin tests.** Delayed reading intradermal and/or patch tests have been used for many years in the evaluation of nonimmediate reactions to BLs, particularly to penicillins, with a sensitivity ranging from 2.6% (positive responses to patch tests in eight out of 298 patients tested with phenoxymethyl penicillin) (69) to 37.8% (positive responses to patch tests and/or delayed reading intradermal tests with penicillins in 98 out of 259 patients with nonimmediate reactions to penicillins) (70). The general rules recommended in the previous ENDA document (5) and the diagnostic algorithm (Fig. 3) are still valid.

As the original review article (5) was written, new evidence suggests that skin test sensitivity is lower than previously reported. In the studies by Lammintausta et al. (69) and Trcka et al. (71), AX gave positive reactions to patch tests in 10 (4%) out of 247 patients, and BP in six (3.9%) of 152. In order to assess more precisely the value of skin testing, a recent study evaluated prospectively 22 subjects who had developed maculopapular exanthema (MPE) or urticarial exanthema after BL intake. Results indicated that after confirmation of the reproducibility of the reaction by DPT, only two (9%) of these 22 subjects had a positive delayed intradermal/patch test (67). Similar results have been found in children (68). This

indicates that in patients with nonimmediate reactions to BLs (MPE or urticarial exanthema), the sensitivity of skin testing is low and DPT may be required to establish the diagnosis. The sensitivity of skin testing may be higher in more severe reactions, such as desquamative exanthemas. Regarding the drugs involved in the reactions, aminopenicillins still play an important role, with almost all patients tolerating BP (66).

With regard to BLs other than penicillins, only a few large studies have been undertaken on skin test sensitivity. In the aforesaid study (69), cephalosporins elicited positive patch-test reactions in 12 (4.1%) out of 290 patients with cutaneous adverse reactions to these BLs, while meropenem caused positive patch-test reactions in one out of two patients. A recent study of hypersensitivity reactions to cephalosporins in children evaluated 105 children with nonimmediate reactions to cephalosporins (72); only one child had a positive immediate response to a skin test with penicillin reagents.

**In vitro tests.** The more widely used *in vitro* test for the evaluation of nonimmediate reactions to BLs is the lymphocyte transformation test (LTT), although it has not been sufficiently validated. The series reported lack adequate numbers of cases, many drugs are involved, and the clinical entities are quite heterogeneous (73). These

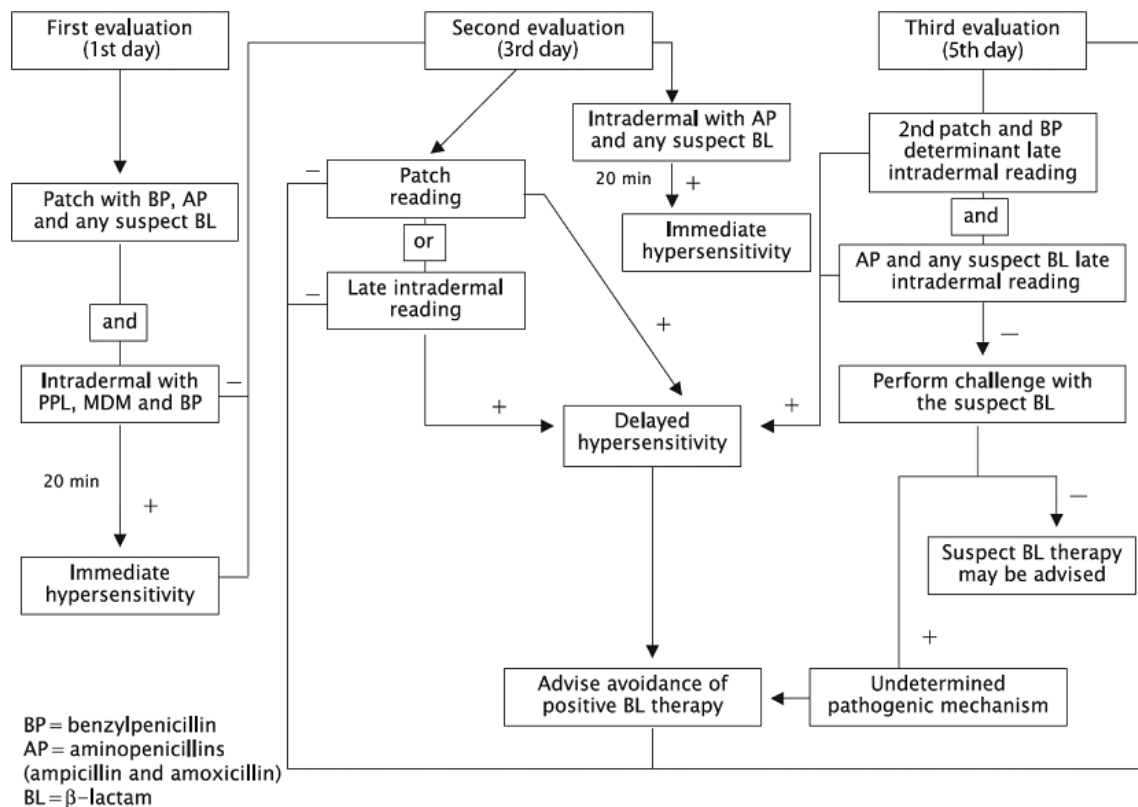


Figure 3. Algorithm for the diagnosis of nonimmediate allergic reactions to betalactams.

studies have found a sensitivity of 74%, but with rather a low specificity (85%) (74–76). Additionally, this test is not available on a large scale and it is still considered a research tool.

A recent study analysing the role of dendritic cells in the enhancement of AX-specific lymphocyte proliferation in patients with nonimmediate reactions to this drug showed that dendritic cells improve the *in vitro* LTT in comparison with other classically used antigen-presenting cells, such as B cells or monocytes. This increased sensitivity of the test was also accompanied by a high specificity. Further studies are needed to confirm these data (77).

Other studies on the generation of T-cell lines, T-cell clones and cytotoxicity have provided information on the involvement of drug-specific T cells (78, 79). However, because of their complex methodology, these *in vitro* studies are only used for research purposes.

**Drug provocation tests.** The ENDA document (5) stated that the DPT was, at that time, the best tool to confirm a causal relationship between drug administration and nonimmediate adverse reactions. It relies on the careful administration of the suspect agent, in a specialized centre under careful clinical observation, paying particular attention to cutaneous symptoms. DPT is contraindicated in patients with severe reactions, such as bullous exanthems, DRESS, Stevens-Johnson syndrome, AGEP and toxic epidermal necrolysis (5). Haematologic reactions, e.g. anaemia, neutropenia or vasculitis, also represent a contraindication.

In some studies, BL-associated cutaneous eruptions – displaying negative results in the allergological work-up – have been provoked with the suspected drugs, with most subjects tolerating the DPT (66–72). Specifically, in the study by Romano et al. (70) only three (2.5%) of the 117 subjects reacted to DPT with the suspect penicillins, whereas in that by Trcka et al. (71) three (3.8%) of the 78 subjects who underwent DPT with BP or AX reacted to these, experiencing exanthematous eruptions. As far as cephalosporins are concerned, in the latter study (71), only one out of the 75 patients with cutaneous eruptions associated with cephalosporins and negative results in allergological tests reacted to DPT with the suspected cephalosporins (cefadroxil or cephalexin). Moreover, in the previously mentioned study involving children (72), 96 out of the 104 subjects with nonimmediate reactions to cephalosporins and negative results in allergological tests underwent DPT; 95 of them tolerated the drugs and one reacted to a paediatric suspension of Cefaclor but tolerated the DPT with a Cefaclor capsule.

However, some patients with negative results in allergological tests react to the DPT, with nonimmediate cutaneous eruptions, particularly maculopapular and

urticarial reactions (67). Because of the decreased skin test sensitivity in nonimmediate reactions to BLs, many patients need a DPT with the culprit drug to be diagnosed.

#### Assessment of cross-reactivity

In nonimmediate allergy to aminopenicillins, both the core structure of the BL and the whole molecule (core structure and the amino-benzyl group of the side-chain) are recognized by T cells (78, 79). However, the amino-benzyl group plays a predominant role; more than 70% of patients with such hypersensitivity display negative responses to BP in intradermal and/or patch tests (66–68). This means that side-chain alterations affect recognition, but that the same side-chains presented by cephalosporin core structures are not recognized. The pattern of cross-reactivity indicates that in the event of a confirmed nonimmediate reaction, as many subjects who respond to aminopenicillins have good tolerance to BP and subjects who respond to cephalosporins may have good tolerance to penicillin derivatives, DPT can help to assess tolerance. In a recent study (71), 69 (97.2%) out of 71 patients with delayed hypersensitivity to aminopenicillins tolerated cephalosporins, such as cefpodoxime, with no amino-benzyl side-chain, and 51 subjects (71.8%) also tolerated phenoxymethyl penicillin. Thus, cross-reactivity between cephalosporins and penicillins seems to be very rare for T-cell reactions.

#### Concluding remarks

As allergic reactions to BLs are the most important cause of immunological reactions to drugs and considering that changes in the number of drugs available and patterns of consumption have modified the sensitivity of the tests, periodical adaptation to these changes is required. This review provides an update to the original ENDA document that gave the guidelines for the evaluation of patients with immediate and nonimmediate hypersensitivity reactions to BLs.

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## References

- Antúnez C, Martín E, Cornejo-García JA, Blanca-Lopez N, R-Pena R, Mayorga C et al. Immediate hypersensitivity reactions to penicillins and other betalactams. *Curr Pharm Des* 2006;**12**:3327–3333.
- Guéant JL, Guéant-Rodriguez RM, Viola M, Valluzzi RL, Romano A. IgE-mediated hypersensitivity to cephalosporins. *Curr Pharm Des* 2006;**12**:3335–3345.
- Blanca M, Cornejo-García JA, Torres MJ, Mayorga C. Specificities of B cell reactions to drugs. The penicillin model. *Toxicology* 2005;**209**: 181–184.
- Torres MJ, Blanca M, Fernandez J, Romano A, de Weck A, Aberer W et al. Diagnosis of immediate allergic reactions to beta-lactam antibiotics. *Allergy* 2003;**58**:961–972.
- Romano A, Blanca M, Torres MJ, Bircher A, Aberer W, Brockow K et al. Diagnosis of nonimmediate reactions to beta-lactam antibiotics. *Allergy* 2004;**59**:1153–1160.
- Levine BB, Ovary Z. Studies of the mechanism of the formation of the penicillin antigen III. The N(D-(benzylpenicilloyl)) group as an antigenic determinant responsible for hypersensitivity to penicillin G. *J Exp Med* 1961;**114**:875.
- Dewdney JM. Immunology of the antibiotics. In: Sela M, editor. *The antigens*, Vol. IV. Academic Press, New York, 1977:114–122.
- Blanca M, Vega JM, García J, Carmona MJ, Terrados S, Miranda A et al. Allergy to amoxicillin with good tolerance to other penicillins. Study of the incidence in patients allergic to betalactams. *Clin Exp Allergy* 1990;**20**:475–481.
- Silviu Dan F, Mc Philips S, Warrington R. The frequency of skin test reactions to side chain penicillin determinants. *J Allergy Clin Immunol* 1993;**91**:694–701.
- Romano A, Mayorga C, Torres MJ, Artesani MC, Suau R, Pérez E et al. Immediate allergic reactions to cephalosporins: cross-reactivity and selective responses. *J Allergy Clin Immunol* 2000;**106**:1177–1183.
- Romano A, Quarantino D, Aimone-Gastin I, Mayorga C, Papa G, Venuti A et al. Cephalosporin allergy: characterization of unique and cross-reacting cephalosporin antigens. *Int J Immunopathol Pharmacol* 1997;**10**:187–191.
- Romano A, Blanca M, Mayorga C, Venuti A, Gasbarrini G. Immediate hypersensitivity to penicillins. Studies on Italian subjects. *Allergy* 1997;**52**:89–93.
- Blanca M, Mayorga C, Torres MJ, Warrington R, Romano A, Demoly P et al. Side chain specific reactions to betalactams: fourteen years later. *Clin Exp Allergy* 2002;**32**:192–197.
- Antunez C, Blanca-Lopez N, Torres MJ, Mayorga C, Perez-Inestrosa E, Montañez MI et al. Immediate allergic reactions to cephalosporins: Evaluation of cross-reactivity with a panel of penicillins and cephalosporins. *J Allergy Clin Immunol* 2006;**117**:404–410.
- Romano A, Torres MJ, Fernandez J, Vega JM, Mayorga C, García J et al. Allergic reactions to ampicillin. Studies on the specificity and selectivity in subjects with immediate reactions. *Clin Exp Allergy* 1997;**27**:1425–1431.
- Romano A, Guéant-Rodriguez RM, Viola M, Amoghly F, Gaeta F, Guéant JL. Diagnosing immediate reactions to cephalosporins. *Clin Exp Allergy* 2005;**35**:1234–1242.
- Sanchez-Sancho F, Perez-Inestrosa E, Suau R, Montañez MI, Mayorga C, Torres MJ et al. Synthesis, characterization and immunochemical evaluation of cephalosporin antigenic determinants. *J Mol Reognit* 2003;**16**:148–156.
- Perez-Inestrosa E, Suau R, Montañez MI, Rodríguez R, Mayorga C, Torres MJ et al. Cephalosporin chemical reactivity and its immunological implications. *Curr Opin Allergy Clin Immunol* 2005;**5**:323–330.
- Baldo BA, Zhao Z, Pham NH. Antibiotic allergy: immunochemical and clinical considerations. *Curr Allergy Asthma Rep* 2008;**8**:49–55.
- Levine BB, Redmond AP. Minor haptenic determinant specific reagins of penicillin hypersensitivity in man. *Int Arch Allergy Appl Immunol* 1969;**35**:445–455.
- Torres MJ, Padial A, Mayorga C, Fernández T, Sanchez-Sabate E, Cornejo-García JA et al. The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. *Clin Exp Allergy* 2004;**34**:1768–1775.
- Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF, Bock SA, Branum A et al. Second symposium on the definition and management of anaphylaxis: summary report-Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol* 2006;**117**: 391–397.
- Demoly P, Kropf R, Bircher A, Pichler WJ. Drug hypersensitivity questionnaire. *Allergy* 1999;**54**:999–1003.
- Executive summary of disease management of drug hypersensitivity: a practice parameter. Joint Task Force on Practice Parameters, the American Academy of Allergy, Asthma and Immunology, the American Academy of Allergy, Asthma and Immunology, and the Joint Council of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* 1999;**83**:665–700.
- Rodríguez-Bada JL, Montañez MI, Torres MJ, Mayorga C, Canto G, Perez-Inestrosa E et al. Skin testing for immediate hypersensitivity to betalactams: comparison between two commercial kits. *Allergy* 2006;**61**: 947–951.
- Romano A, Viola M, Bousquet PJ, Gaeta F, Valluzzi R, Caruso C et al. A comparison of the performance of two penicillin reagent kits in the diagnosis of beta-lactam hypersensitivity. *Allergy* 2007;**62**:53–58.
- Nolan RC, Puy R, Deckert K, O'Hehir RE, Douglass JA. Experience with a new commercial skin testing kit to identify IgE-mediated penicillin allergy. *Intern Med J* 2008;**38**:357–367.
- Macy E, Line CK, Goldberg B. Penicillin skin testing using a complete panel of major and minor determinants. *J Allergy Clin Immunol* 2008;**121**:S193.
- Romano A, Bousquet-Rouanet L, Viola M, Gaeta F, Demoly P, Bousquet JP. Benzylpenicillin skin testing is still important in diagnosing immediate hypersensitivity reactions to penicillins. *Allergy* 2008; in press.
- Fernandez-Rivas M, Perez Carral C, Cuevas M, Marti C, Moral A, Senet CJ. Selective allergic reactions to clavulanic acid. *J Allergy Clin Immunol* 1995;**95**:748–750.
- Torres MJ, Romano A, Mayorga C, Moya MC, Guzman AE, Reche M et al. Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. *Allergy* 2001;**56**: 850–856.
- Bousquet PJ, Co-Minh HB, Arnoux B, Daures JP, Demoly P. Importance of mixture of minor determinants and benzylpenicilloyl poly-L-lysine skin testing in the diagnosis of beta-lactam allergy. *J Allergy Clin Immunol* 2005;**115**:1314–1316.

33. Jost BC, Wedner HJ, Bloomberg GR. Elective penicillin skin testing in a pediatric outpatient setting. *Ann Allergy Asthma Immunol* 2006;**97**: 807–812.
34. Parker PJ, Parrinello JT, Condemi JJ, Rosenfeld SI. Penicillin resensitization among hospitalized patients. *J Allergy Clin Immunol* 1991;**88**:213–217.
35. Pichichero ME, Pichichero DM. Diagnosis of penicillin, amoxicillin, and cephalosporin allergy: reliability of examination assessed by skin testing and oral challenge. *J Pediatr* 1998;**132**: 137–143.
36. Matheu V, Pérez-Rodríguez E, Sánchez-Machin I, García-Robaina JC, de la Torre Morin F. Importance of repeat testing in the diagnosis of penicillin allergy. *Br J Dermatol* 2006;**154**:198.
37. Goldberg A, Confino-Cohen R. Skin testing and oral penicillin challenge in patients with a history of remote penicillin allergy. *Ann Allergy Asthma Immunol* 2008;**100**:37–43.
38. Antúnez C, Fernández T, Blanca-Lopez N, Torres MJ, Mayorga C, Canto G et al. IgE antibodies to betalactams: relationship between the triggering hapten and the specificity of the immune response. *Allergy* 2006;**61**:940–946.
39. Co Minh HB, Bousquet PJ, Fontaine C, Kvedariene V, Demoly P. Systemic reactions during skin tests with betalactams: a risk factor analysis. *J Allergy Clin Immunol* 2006;**117**:466–468.
40. Blanca M, Mayorga C, Torres MJ, Reche M, Moya MC, Rodríguez JL et al. Clinical evaluation of Pharmacia CAP System RAST FEIA amoxicilloyl and benzylpenicilloyl in patients with penicillin allergy. *Allergy* 2001;**56**: 862–870.
41. Sanz ML, Gamboa PM, Antépara I, Uasuf C, Vila L, García-Avilés C et al. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy* 2002;**32**:277–286.
42. Fontaine C, Mayorga C, Bousquet PJ, Arnoux B, Torres MJ, Blanca M et al. Relevance of the determination of serum-specific IgE antibodies in the diagnosis of immediate beta-lactam allergy. *Allergy* 2007;**62**:47–52.
43. Fernandez TD, Torres MJ, Blanca-Lopez N, Rodríguez-Bada JL, Gomez E, Canto G et al. Negativization rates of IgE radioimmunoassay and basophil activation test in immediate reactions to penicillins. *Allergy* 2008; in press.
44. Messaad D, Sahla H, Benahmed S, Godard P, Bousquet J, Demoly P. Drug provocation tests in patients with a history suggesting an immediate drug hypersensitivity reaction. *Ann Intern Med* 2004;**140**:1001–1006.
45. Bousquet PJ, Pipet A, Bousquet-Rouanet L, Demoly P. Oral challenges are needed in the diagnosis of beta-lactam hypersensitivity. *Clin Exp Allergy* 2008;**38**:185–190.
46. Pichichero ME. Use of selected cephalosporins in penicillin-allergic patients: a paradigm shift. *Diagn Microbiol Infect Dis* 2007;**57**:13S–18S.
47. Torres MJ, Blanca-Lopez N, Martin E, Mayorga C, Rodríguez-Bada JL, Doña I et al. Cross-reactivity between penicillins and cephalosporins with the same side chain: amoxicillin and cefadroxil. *J Allergy Clin Immunol* 2007;**119**:38.
48. Audicana M, Bernaola G, Urrutia I, Echechipia S, Gastaminza G, Muñoz D et al. Allergic reactions to betalactams: studies in a group of patients allergic to penicillin and evaluation of cross-reactivity with cephalosporin. *Allergy* 1994;**49**:108–113.
49. Romano A, Guéant-Rodríguez RM, Viola M, Pettinato R, Guéant JL. Cross-reactivity and tolerability of cephalosporins in patients with immediate hypersensitivity to penicillins. *Ann Intern Med* 2004;**141**:16–22.
50. Baldo BA. Penicillins and cephalosporins as allergens – structural aspects of recognition and cross-reactions. *Clin Exp Allergy* 1999;**29**:744–749.
51. Miranda A, Blanca M, Vega JM, Moreno F, Carmona MJ, García JJ et al. Cross-reactivity between a penicillin and a cephalosporin with the same side chain. *J Allergy Clin Immunol* 1996;**98**:671–677.
52. Blanca M, Fernandez J, Miranda A, Terrados S, Torres MJ, Vega JM et al. Cross-reactivity between penicillins and cephalosporins: clinical and immunologic studies. *J Allergy Clin Immunol* 1989;**83**:381–385.
53. Romano A, Viola M, Gueant-Rodríguez RM, Gaeta F, Pettinato R, Guéant JL. Imipenem in patients with immediate hypersensitivity to penicillins. *N Engl J Med* 2006;**354**:2835–2837.
54. Romano A, Viola M, Guéant-Rodríguez RM, Gaeta F, Valluzzi R, Guéant JL. Brief communication: tolerability of meropenem in patients with IgE-mediated hypersensitivity to penicillins. *Ann Intern Med* 2007;**146**: 266–269.
55. Atanasković-Marković M, Gaeta F, Medjo B, Viola M, Nestorović B, Romano A. Tolerability of meropenem in children with IgE-mediated hypersensitivity to penicillins. *Allergy* 2008;**63**:237–240.
56. Vega JM, Blanca M, García JJ, Miranda A, Carmona MJ, García A et al. Tolerance to aztreonam in patients allergic to beta-lactam antibiotics. *Allergy* 1991;**46**:196–202.
57. Pérez Pimiento A, Gómez Martínez M, Mínguez Mena A, Trampal González A, de Paz Arranz S, Rodríguez Mosquera M. Aztreonam and ceftazidime: evidence of in vivo cross allergenicity. *Allergy* 1998;**53**:624–625.
58. Cohen AD, Friger M, Sarov B, Halevy S. Which intercurrent infections are associated with maculopapular cutaneous drug reactions? A case-control study. *Int J Dermatol* 2001;**40**: 41–44.
59. Yawalker N, Karlen S, Eqli F, Brand CU, Qabee HU, Dichlee WJ et al. Infiltration of cytotoxic T cells in drug-induced cutaneous eruptions. *Clin Exp Allergy* 2000;**30**:847–855.
60. Blanca M, Posadas S, Torres MJ, Leyva L, Mayorga C, Gonzalez L et al. Expression of the skin-homing receptor in peripheral blood lymphocytes from subjects with nonimmediate cutaneous allergic drug reactions. *Allergy* 2000;**55**:998–1004.
61. Posadas SJ, Padial A, Torres MJ, Mayorga C, Leyva L, Sanchez E et al. Delayed reactions to drugs show levels of perforin, granzyme B, and Fas-L to be related to disease severity. *J Allergy Clin Immunol* 2002;**109**: 155–161.
62. Fernandez TD, Mayorga C, Torres MJ, Cornejo-García JA, López S, Chaves P et al. Cytokine and chemokine expression in the skin from patients with maculopapular exanthema to drugs. *Allergy* 2008;**63**:712–719.
63. Padial MA, Alvarez-Ferreira J, Tapia B, Blanco R, Mañas C, Blanca M et al. Acute generalized exanthematous pustulosis associated with pseudoecephedrine. *Br J Dermatol* 2004;**150**: 139–142.
64. Nassif A, Bensussan A, Boumsell L, Deniaud A, Moslehi H, Wolkenstein P et al. Toxic epidermal necrolysis: effector cells are drug-specific cytotoxic T cells. *J Allergy Clin Immunol* 2004;**114**:1209–1215.
65. Shiohara T, Kano Y. A complex interaction between drug allergy and viral infection. *Clin Rev Allergy Immunol* 2007;**33**:124–133.

66. Romano A, Di Fonso M, Papa G, Pietrantonio F, Federico F, Fabrizi G et al. Evaluation of adverse cutaneous reactions to aminopenicillins with emphasis on those manifested by maculopapular rashes. *Allergy* 1995;**50**:113–118.
67. Padial A, Antunez C, Blanca-Lopez N, Fernandez TD, Cornejo-Garcia JA, Mayorga C et al. Non-immediate reactions to beta-lactams: diagnostic value of skin testing and drug provocation test. *Clin Exp Allergy* 2008;**38**:822–828.
68. Blanca-Lopez N, Zapatero L, Alonso E, Torres MJ, Fuentes V, Martinez-Molero MI et al. Skin testing and drug provocation tests in the diagnosis of non-immediate reactions to aminopenicillins in children. *Allergy*, in press.
69. Lammintausta K, Kortekangas-Savolainen O. The usefulness of skin tests to prove drug hypersensitivity. *Br J Dermatol* 2005;**152**:968–974.
70. Romano A, Viola M, Mondino C, Pettinato R, Di Fonso M, Papa G et al. Diagnosing nonimmediate reactions to penicillins by in vivo tests. *Int Arch Allergy Immunol* 2002;**129**:169–174.
71. Trcka J, Seitz CS, Bröcker EB, Gross GE, Trautmann A. Aminopenicillin-induced exanthema allows treatment with certain cephalosporins or phenoxymethyl penicillin. *J Antimicrob Chemother* 2007;**60**:107–111.
72. Romano A, Gaeta F, Valluzzi RL, Alonzi C, Viola M, Bousquet JP. Diagnosing hypersensitivity reactions to cephalosporins in children. *Pediatrics* 2008;**122**:521–2.
73. Rosso R, Mattiacci G, Bernardi ML, Guazzi V, Zaffiro A, Bellegrandi S et al. Very delayed reactions to  $\beta$ -lactam antibiotics. *Contact Dermat* 2000;**42**:293–295.
74. Nyfeler B, Pichler WJ. The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. *Clin Exp Allergy* 1997;**27**:175–181.
75. Schnyder B, Pichler WJ. Skin and laboratory tests in amoxicillin- and penicillin-induced morbilliform skin eruption. *Clin Exp Allergy* 2000;**30**:590–595.
76. Luque I, Leyva L, Torres MJ, Rosal M, Mayorga C, Segura JM et al. *In vitro* T-cell responses to  $\beta$ -lactam drugs in immediate and nonimmediate allergic reactions. *Allergy* 2001;**56**:611–618.
77. Rodriguez-Pena R, Lopez S, Mayorga C, Antunez C, Fernandez TD, Torres MJ et al. Potential involvement of dendritic cells in delayed-type hypersensitivity reactions to beta-lactams. *J Allergy Clin Immunol* 2006;**118**:949–956.
78. Padovan E, Bauer T, Tongio MM, Kalbacher H, Weltzien HU. Penicilloyl peptides are recognized as T cell antigenic determinants in penicillin allergy. *Eur J Immunol* 1997;**27**:1303–1307.
79. Mauri-Hellweg D, Zanni M, Frei E, Bettens F, Brander C, Mauri D et al. Cross-reactivity of T cell lines and clones to beta-lactam antibiotics. *J Immunol* 1996;**157**:1071–1079.